FURROWING IN FLATTENED SEA URCHIN EGGS

ALLAN C. SCOTT [1]

Colby College, Waterville, Maine and Stazione Zoologica, Naples, Italy

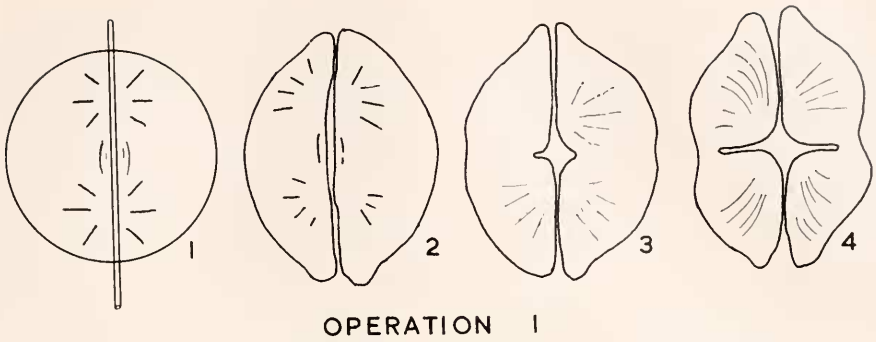
Fifty years ago Yatsu (1908, 1910, 1912) operated with hand-held instruments on cleaving eggs of the nemertine, Cerebratulus and the ctenophore, Beroë. He was able to remove large polar segments from cleaving eggs without interfering with the process. He found that once started in division, the cells demonstrated the greatest pertinacity to divide in spite of operations of the grossest sort. In the years since, many workers have used microdissection techniques on dividing cells: Chambers (1924, 1938) for many exploratory dissections; Dan (1943) to test his astral theory of cytokinesis; Mitchison (1953) in connection with the surface expansion theory; and Hiramoto (1957) to measure the thickness of the cortex during the division cycle. We have used the method to test whether or not "isolated" pieces of furrow cortex undergo contraction. We used the swollen flattened egg as our experimental material because when flattened the mitotic figure is clear, and because cuts can be placed with precision. We have concluded from observations reported here that the position of the furrow is fixed only after elongation has begun, that its position is not necessarily related to the equatorial plane, and that the direction of constriction may be completely altered during the division.

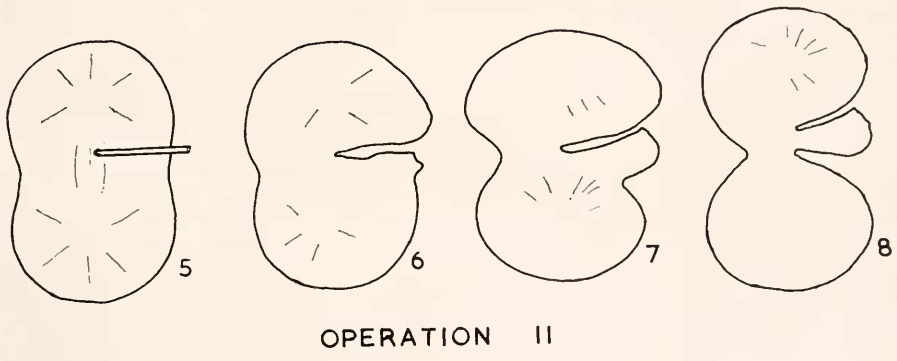
It is not yet possible to adopt any theory of the mechanism of cell division without the gravest reservations. The recent review by Swann and Mitchison (1958) lists eight theories, each one designed to explain a limited set of observations; no over-all theory has emerged, indeed two quite opposite schemes have been proposed to account for the surface changes first observed by Dan, Yanagita and Sugiyama in 1937. According to one of these theories the non-furrow surface expands actively during division (Mitchison, 1952; Swann, 1952); the other theory holds that the furrow cortex contracts actively (Marsland and Landau, 1954). No experiment has yet been reported which will test these two alternatives in a critical way; however, in a previous paper (Scott, 1946), we presented evidence for the continued contraction of the long gelated stalks which occur when eggs cleave in hypotonic sea water. In this paper we present further evidence for the contraction hypothesis.

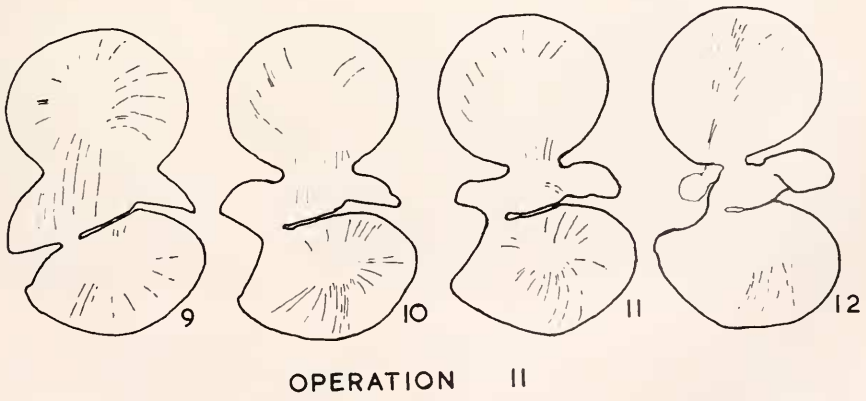
Material and Methods

Extreme flattening of the sea urchin egg suppresses cytoplasmic division but the division of flattened eggs is facilitated when they are swollen in 60% sea

[1] This investigation was carried out during tenure of a National Science Foundation Fellowship. We are indebted to the Stazione Zoologica, and especially to its Director, for facilities furnished and for the general spirit of helpfulness.







FIGURES 1–12. Operation I, Figures 1–4, hemisection of the entire egg followed by division of each half egg. Operation II, Figures 5–8 and 9–12, near abscission of large polar segments with completion of furrowing.

water. This seems to be due to a more fluid endoplasm which offers less resistance to the constricting furrow.

The eggs of *Paracentrotus lividus* at Naples are approximately 88 micra in diameter. In 60% sea water they swell to a nearly constant diameter of 100 micra in ten minutes and so increase their volume by 47%. If the swollen eggs are flattened to a diameter of 150 micra their thickness is reduced to approximately 30 micra.

The general technique used was as follows: Eggs were obtained from excised ovaries and were washed twice by settling and decantation. Since there is great variability in the ripeness of different females, tests were made to find a female whose eggs produced high fertilization membranes in at least 95% of the eggs sampled. If control samples of any egg batch failed to cleave with at least 95% normal first cleavage, the egg batch was discarded. The fertilization membranes were removed at three minutes by shaking. At 65 to 70 minutes the eggs were removed to 60% sea water and prepared for microdissection in a hanging drop on a very clean, previously flamed, slide. The degree of flattening was regulated by withdrawing the appropriate amount of the dilute sea water with a fine pipette. Most of the operations were slit punctures made with the flat side of a microneedle. Observations were made on the flattened eggs still mounted in the moist chamber. The manipulator was made by De Fonbrun and the needles were drawn with a De Fonbrun microforge.

Results

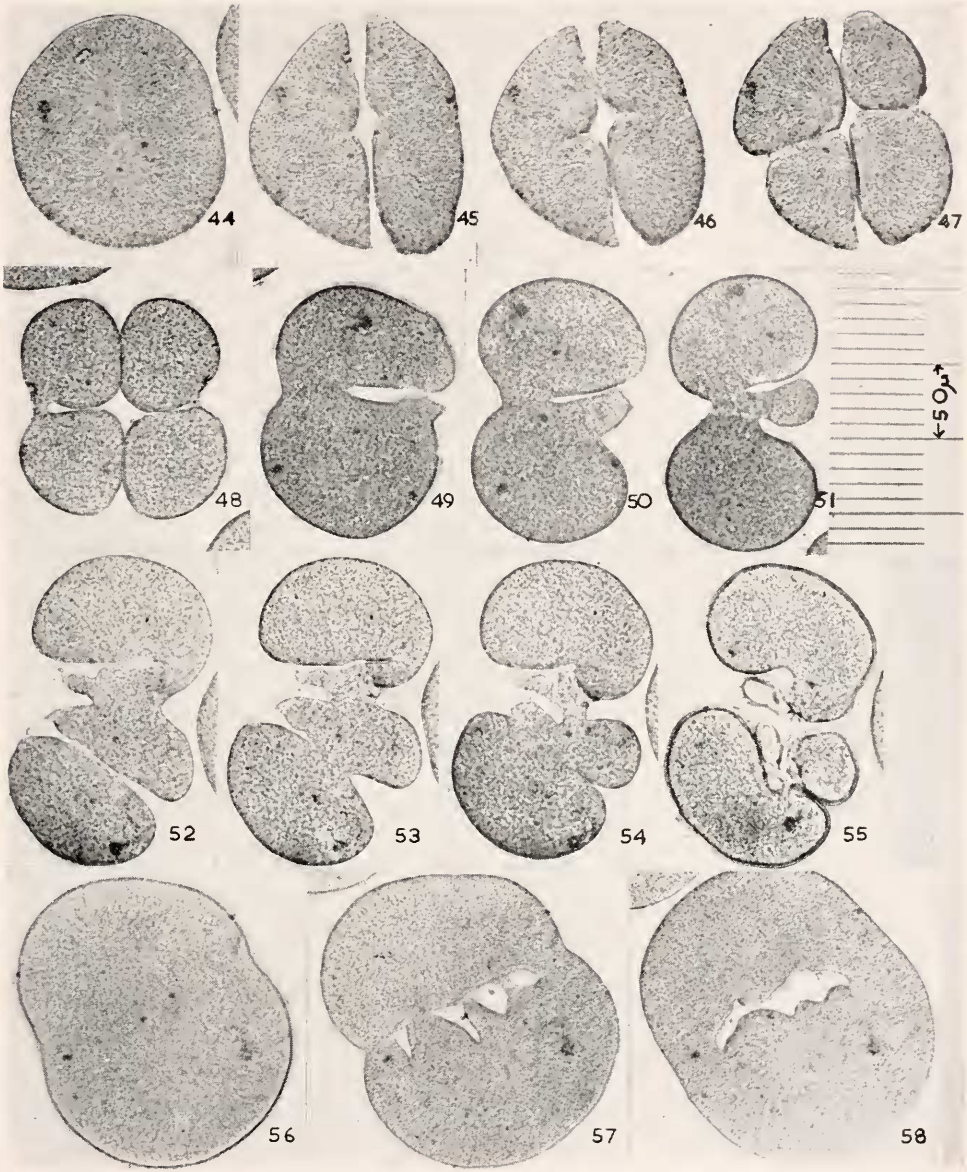
Ten different experiments were performed on the flattened egg. The operations reported involve only the period from mid-diaster, that is, about ten minutes before the first cleavage begins, through the furrowing period. The ten operations are designated I through X and are individually described in the following paragraphs and figured in the accompanying line drawings, Figures 1–43 and photographs, Figures 44–80.

Operation I, hemisection of the entire egg, Figures 1–4 and 44–48:

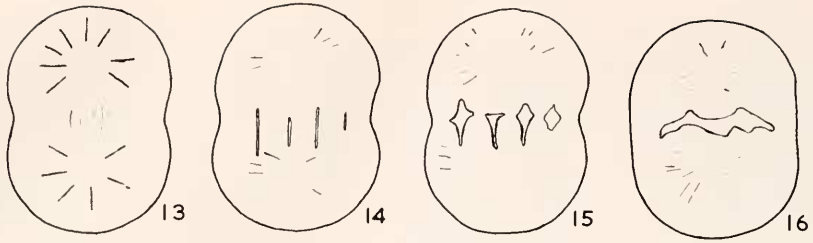
It is surprising to find that when eggs are bisected with a cut which divides the amphiaster neatly through its long axis, both fragments cleave. Each fragment retains visible parts of the half asters and the half spindle. Sometimes the furrow cuts in symmetrically towards the new median longitudinal axis of the half egg or alternatively it cuts out from the plane of hemisection. Yatsu reported that large segments cut from the "side" of the dividing egg completed the furrowing but this observation of cleaving hemisected eggs has a certain elegance when performed on a flattened egg, in that the flatness allows a precise bisection of egg and amphiaster.

Operation II, abscission or near abscission of polar segments, Figures 5–8, 9–12, and 49–55:

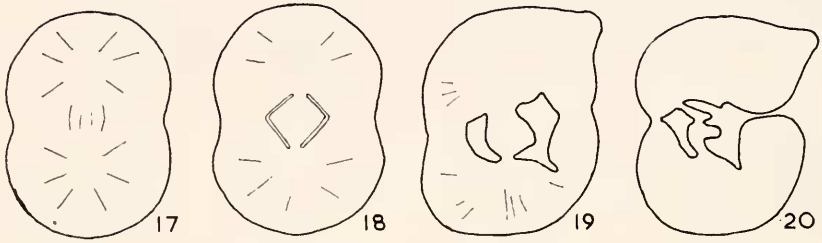
It is possible to cut away one or both poles at the cylinder stage, removing as much as two-thirds of the egg substance, and still get successful furrowing. Neither the position of the furrow nor its course is altered by the operation. This experi-



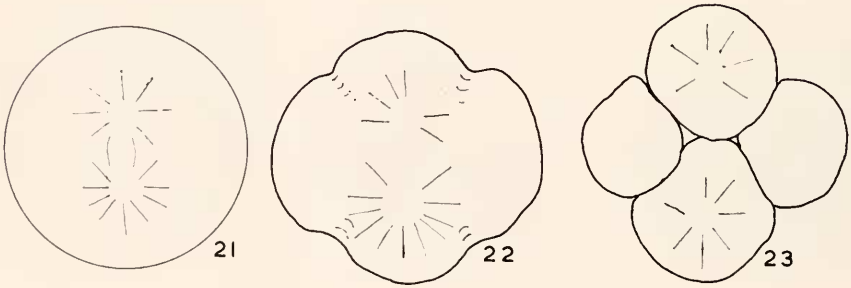
FIGURES 44–58. Photographs illustrating Operations I–III. Operation I, Figures 44–48, hemisection of entire egg with continued furrowing. Operation II, Figures 49–51, continued furrowing after near abscission of one pole. Operation II, Figures 52–55, continued furrowing after near abscission of both poles. Operation III, Figures 56–58, rapid opening of slit perforations (see text). Figure 57 is a few seconds after Figure 56; Figure 58 is 5 minutes later.



OPERATION III



OPERATION IV



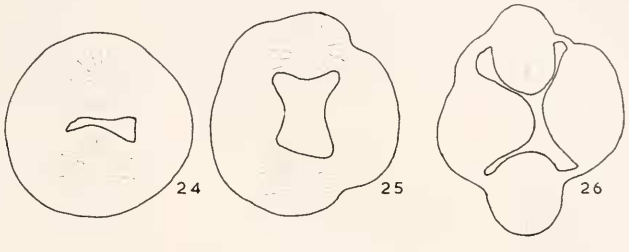
OPERATION V

FIGURES 13–23. Operation III, Figures 13–16, longitudinal slits in the furrow showing the immediate opening-out of the slits and the completion of three internal furrows. Operation IV, Figures 17–20, near isolation of a furrow segment, Figure 18, followed by shrinkage of the segment, Figures 19 and 20. Operation V, Figures 21–23, cleavage of a flattened diastral egg to four cells; two cells with, and two without, aster and nucleus.

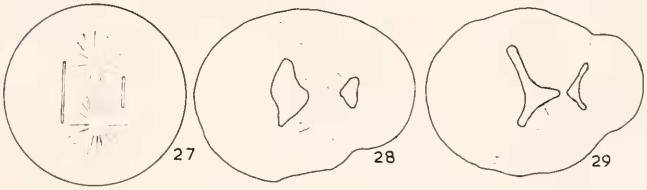
ment separates the expanding polar surface from the rest of the egg, during a considerable part of the time that it would be expanding in the unoperated egg, without interfering with cleavage.

Operation III, longitudinal slits in the furrow, Figures 13–16 and 56–58:

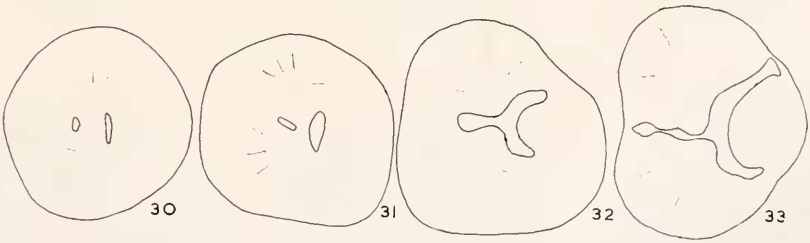
During this operation, the egg is flattened in the cylinder stage and one or more slit perforations are made across the furrow with the long axis of the slit parallel



OPERATION VI

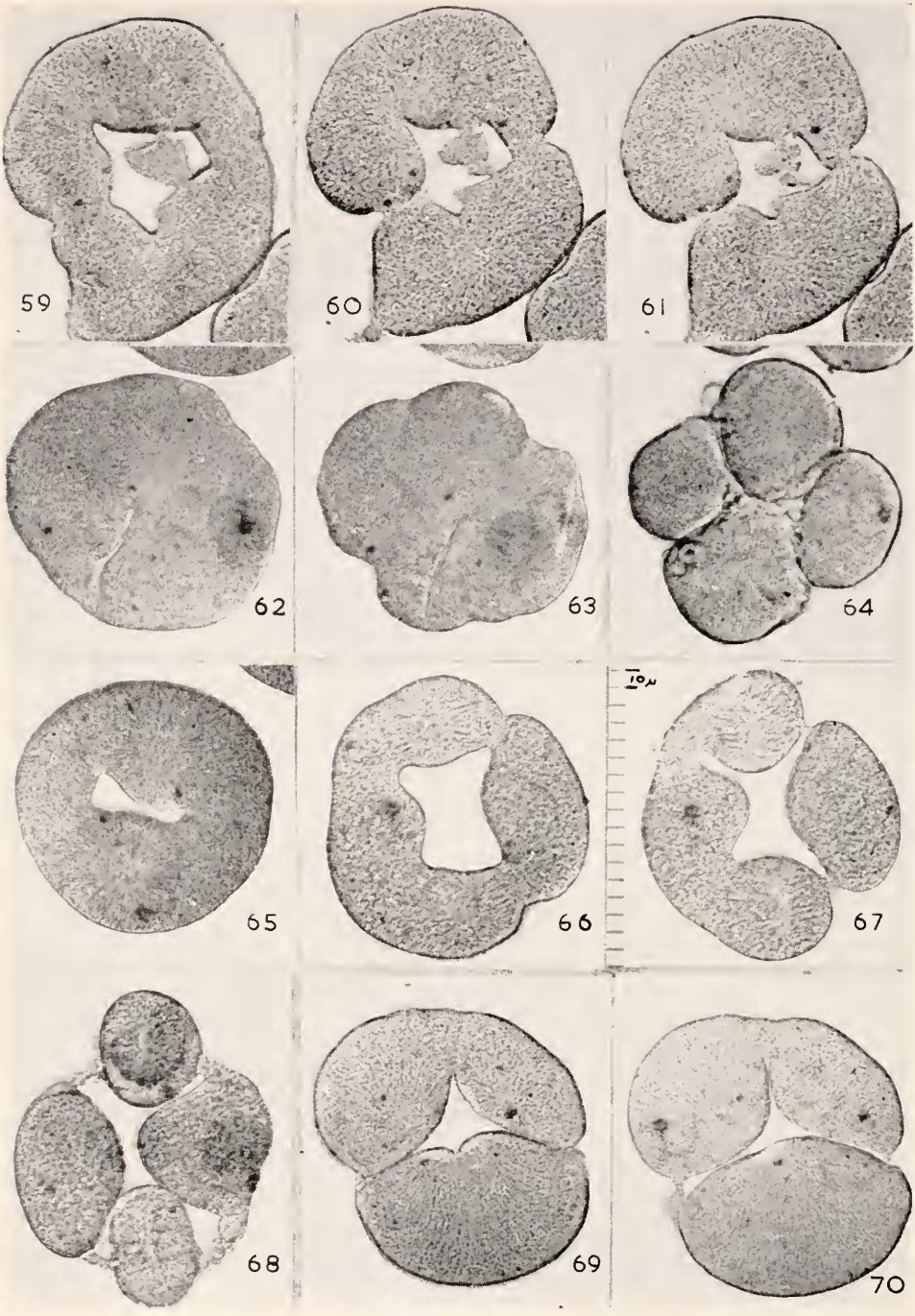


OPERATION VII CASE I



OPERATION VII CASE II

FIGURES 24–33. Operation VI, Figures 24–26, transection of the spindle; development of four furrows and cleavage to four cells, two cells without asters and without nucleus. Operation VII, Case I, Figures 27–29, slit perforations lateral to the spindle; five furrows produce four cells, two without aster or nucleus. Operation VII, Case II, Figures 30–33, slit perforations lateral to the spindle; three cells are formed, one without aster or nucleus.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to the long axis of the egg. The slits immediately open in a way that gives evidence that the furrow ring is a region of tension in flattened eggs. Within seconds the slits open and multiple furrows produced gradually cut through the narrowing stalks. This operation makes cleavage possible in eggs that would otherwise be stalled at the cylinder stage; it is apparently easier for an egg to furrow through several small stalks than through one big one.

Operation IV, near isolation of a furrow segment, Figures 17–20 and 59–61:

In this operation four perforating cuts are made as shown in Figure 18, so that a central plug of egg substance, the surface of which is virtually all furrow cortex, is isolated from the rest of the egg. Only slender stalks connect the plug with the sub-furrow region. During the next few minutes the whole semi-isolated plug shrinks in volume; meanwhile its contained endoplasm flows through the attachments into the main part of the cell. We consider this to result from an active contraction of the entire surface of the plug; it appears to be incompatible with the membrane expansion theory.

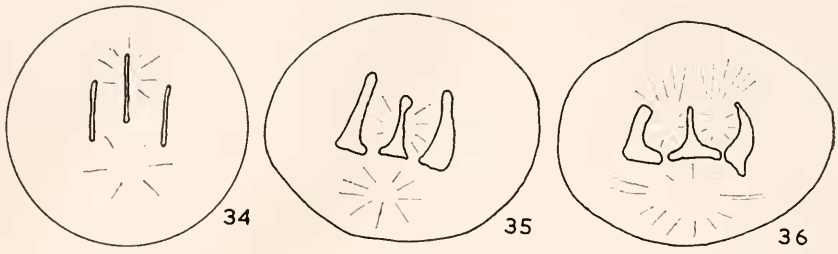
Operation V, flattening without microdissection, Figures 21–23 and 62–64:

When eggs in the middle diaster are swollen, flattened but otherwise unoperated, they may cleave directly to four cells, two of which contain asters and nuclei and two that do not. The expected single equatorial furrow does not develop; instead, four areas of furrowing move in from the periphery following the tips of the astral rays. In two cases the four-celled embryo was followed through a second cleavage during which the nucleated cells divided again while the anucleate ones failed to divide; the latter did, however, each develop a large monaster during the "division phase," which is the more interesting since they were patently devoid of any part of the amphiaster during the first division.

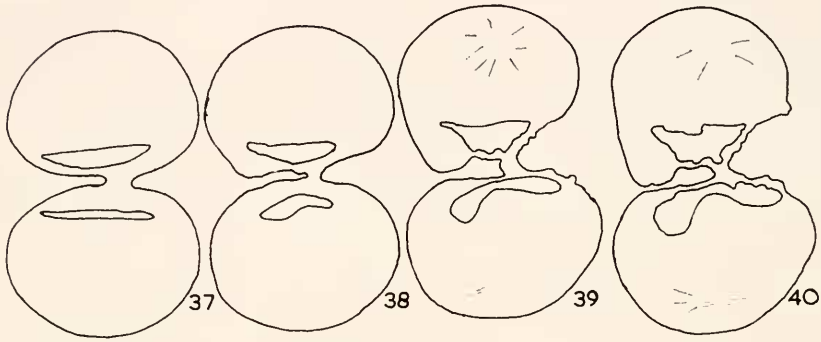
Operation VI, equatorial transection of the spindle I, Figures 24–26 and 65–68:

When one makes a slit-perforation across the equator of the spindle of a mid-diaster stage the perforation soon opens up to form a large hole. The egg then divides directly to four cells, two with nuclei and two without. This is not an occasional result but occurs quite regularly following the transection operation. In all cases with central perforations the furrowing tends to be centrifugal; the furrow cuts out from the hole.

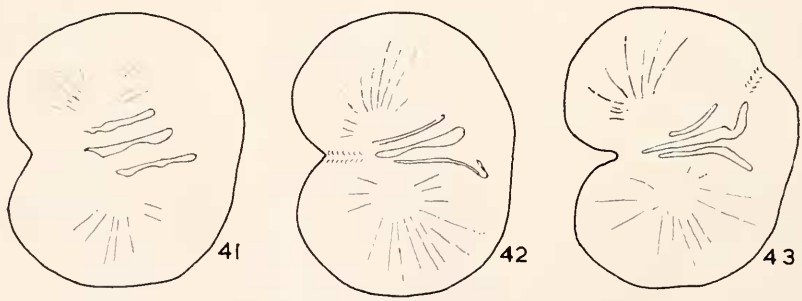
FIGURES 59–70. Photographs illustrating changes after Operations IV, V, VI, and VIII. Operation IV, 59–61, isolation of an island of furrow substance at the cylinder stage showing shrinkage of the furrow island. Operation V, Figures 62–64, flattening without dissection of the furrow with subsequent division of the diastral cell to four cells; two with aster and nucleus and two without. Operation VI, Figures 65–68, transection of spindle of a diastral cell followed by cleavage to four cells; two with an aster and a nucleus and two without. Operation VIII, Figures 69 and 70, bisection of one aster with continued furrowing in the furrow plane but no furrowing between the halves of the divided aster.



OPERATION VIII



OPERATION IX



OPERATION X

FIGURES 34–43. Operation VIII–X, Figures 34–36, bisection of an aster; each half aster forms a spherical aster. Operation IX, Figures 37–40, isolation of walls of completed furrow by means of slit perforations. The tubes of cortex thus formed subsequently shrink in diameter. Operation X, Figures 41–43, isolation of the early furrow cortex by means of slit perforations across the spindle axis. The cortical tubes formed shrink in diameter. Figure 41, immediately after the operation; Figure 43 five minutes later.

Operation VII, slits lateral to the spindle, Case I, Figures 27–29 and 71–73:

Four daughter cells are formed when paired perforations are made on opposite sides of the amphiaster. First there is a furrowing across the spindle equator; then four non-spindle furrows work out centrifugally from the ends of the perforations. In this case as in Operation VI two of the four daughter cells are anucleate.

Operation VII, slits lateral to the spindle, Case II, Figures 30–33:

In some cases three furrows form instead of four, producing two cells with nuclei and one without.

Operation VIII, subdivision of an aster, Figures 34–36 and 69–70:

One or both asters may be bisected with a perforating cut which, if placed as indicated in these figures, will have its inner end drawn out in the furrowing. The half asters become asters of smaller size when bisected and each one has a center from which rays diverge more or less symmetrically. All slits made in the vicinity of asters conform to the sphericity of the aster; apparently even subdivided asters tend to be spherical structures.

Operation IX, "isolation" of late furrow cortex, Figures 37–40 and 74–77:

It is possible to isolate the walls of the completed furrow from the rest of the egg by making two perforations across the spindle axis close to the furrow; this operation forms the furrow walls into tubes which shrink in diameter in the same way as the cleavage stalk.

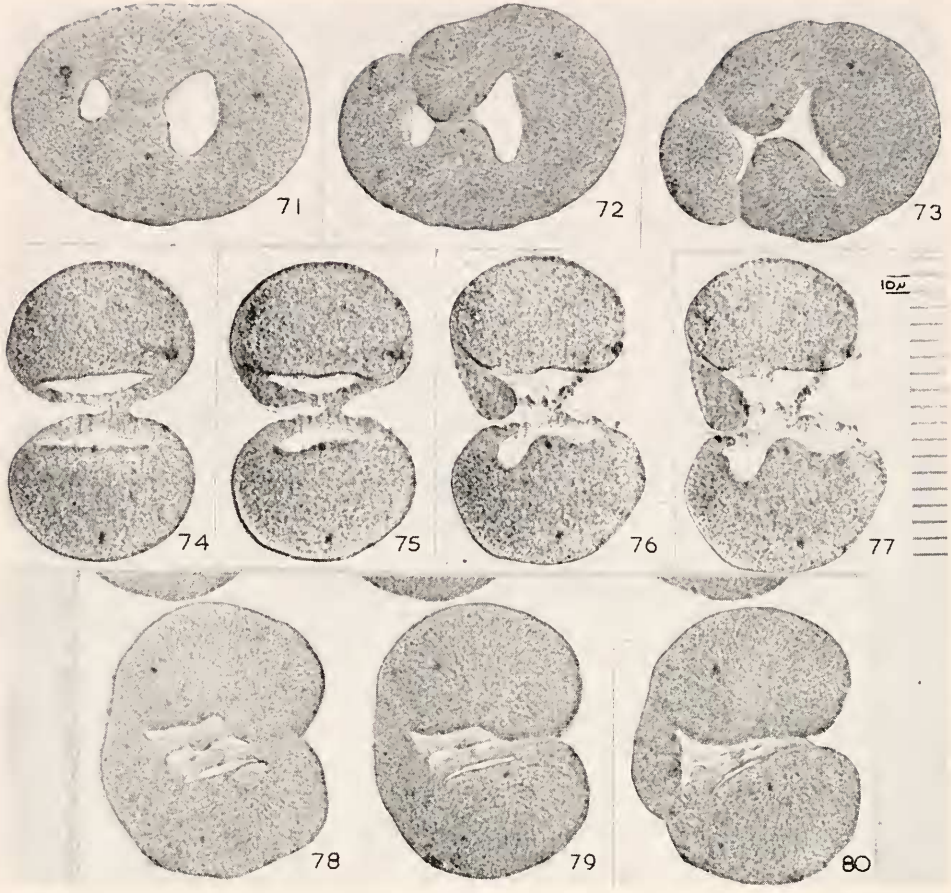
Operation X, "isolation" of the early furrow cortex, Figures 41–43 and 78–80:

In this case the eggs used had just begun to cleave. Two or three slit perforations placed transversely across the mid region isolate tubes of furrow cortex which diminish in diameter like furrow stalks, but their direction of constriction is quite different from the presumptive one.

Discussion

Contraction

We have taken the position that the weight of evidence now favors the theory that cell cleavage occurs by active, cortical contraction. The "proof" will be more compelling if a contractable substance can be extracted from the cell cortex as it can be from muscle. Indeed such proof may be near, for Hoffmann-Berling (1954, 1960) has reported the extraction of a contractile protein from fibroblasts and from sarcoma cells which may be responsible for the furrowing *in vivo* and in his glycerine-water-extracted telophase cell models. One of the strongest indications that the cortex contracts is the shrinkage of the "furrow islands" reported in this study which, although isolated early in furrowing, shrink progressively to become thin strands. In other experiments we produced tubes of furrow cortex



FIGURES 71–80. Photographs illustrating operations VII, IX, and X. Operation VII, Case I, Figures 71–73, slits lateral to the spindle; five furrows form four cells, two with aster and nucleus and two without. Operation IX, Figures 74–77, isolation of tubes of late furrow cortex followed by shrinkage of the tubes. Operation X, Figures 78–80, isolation of tubes of early furrow cortex followed by shrinkage of the tubes.

by a number of different operations (*cf.* Fig. 18 and Fig. 59), after which only narrow connections joined the tubes to the main mass of the egg, and in all cases the tubes diminished in diameter in the manner of a cleavage stalk. Such phenomena are difficult to explain by the membrane expansion theory (Mitchison, 1952; Swann, 1952) which hypothesizes that the expansion of non-furrow surface causes the shrinkages of the furrow. In our experiments furrow cortex is nearly separated from the non-furrow surface, yet it shrinks very effectively. One could argue that the expansion process reaches into the furrow itself but this view must be discarded, as Swann and Mitchison (1958) have pointed out, because there is no area of expansion in the furrow (Dan and Ono, 1954).

Furrow tension

Our experiment III indicates that a circumferential tension is present around the furrow ring by the time of cell elongation. This is contrary to measurements with the elastimeter (Mitchison and Swann, 1955), for no measurable differences in stiffness were found over the surface of the egg at the cleavage stage. We note here that while the eggs used in our studies were swollen, flattened, and certainly abnormal in many ways, still, they did cleave and almost certainly by the same mechanism as normal eggs. Tension around the equator is, as Mitchison and Swann have pointed out, inconsistent with the expansion theory. In their consideration of the flattened egg they have assumed (Mitchison and Swann, 1955) that the tension produced by flattening disappears before expansion begins. They suggest that this would explain the slower furrowing observed after flattening. In the case of experiment III, however, the tension seems never to fall to "zero," for each slit of a series, made one after the other, opens quickly within seconds, indicating that tension still exists around each smaller furrow. The failure to observe tension differences at cleavage with the elastimeter may perhaps be due to the fact that the endoplasm is a fairly viscous gel which resists being drawn into the instrument. Real differences in the stiffness of the cortex would then be masked. It was found that the general stiffness of the surface increases while the asters are quite small, indicating that the aster is not then affecting the measurement, but the "stiffness" value of thixotropic endoplasm is difficult to assess.

What determines where a furrow will form?

In these experiments we have utilized two stages of division for the most part, the one a mid-diaster stage and the other about ten minutes later, at the beginning of cell elongation. The evidence supports the view that the furrow cortex is unspecified during the earlier period and that a definite band of furrow cortex, with special properties, is determined from the moment of cell elongation. It is not possible to perform operations on elongated eggs which will provoke furrowing in areas outside the presumptive furrow. The furrowing cortex is therefore determined; it can be notched, isolated in islands and variously perforated, but it retains its capacity for involvement in constriction. The position of the furrow is not fixed at mid-diaster since simple spindle transection, for example, produces three or four furrows in regions quite different from the presumptive one. What, then, determines where a furrow will form? It has been almost universally accepted that the position of the furrow is related to the spindle equator; thus polar body formation involves such a relationship, whatever the size of the two cells produced, as does micromere formation, the unequal first cleavage in mollusks and abnormal cases involving multipolar spindles. Now, however, the idea of coincidence of mid-spindle and furrow must be abandoned. In our view furrow formation involves three factors:

1. The presence of an aster (not necessarily two), since furrows always follow an astral boundary.

2. The recognition by the cell of a region of narrowness. Deformed cells (see Figure 67) are curiously "opportunistic" in their ability to exploit a region

of narrowness as a potential furrow path. Perhaps the surface in these isthmian regions is differentially stretched and differentially sensitive to furrow initiation.

3. The presence of some (chemical) initiator which may affect the entire circumference of the potential furrow or which may be limited initially to one region of the circumference acting as a furrow head. It seems necessary to assume that the old concept of "furrow head" (Ziegler, 1898) has some validity since many cases occur in which the furrowing process moves progressively over the surface. Yatsu, for example, demonstrated (1910) that the furrow head would bifurcate after some operations disturbing its presumptive path. We conceive of the furrow head in some of these operated eggs as a sector of contraction involving only a part of the furrow ring, the other sector being passive at first, then progressively involved as contraction spreads around the furrow. We conceive, furthermore, of the whole furrow as being under tension as soon as any part of it contracts. This would explain the deep incursions which occur on one side of some furrows while the other side is barely indented. The problem is to explain how an initiator substance appears at precisely those places where furrows will form, for example, at the ends of the slits in Operation III.

Orientation of contractile units

We have shown that operations on the elongated egg may change the orientation of furrowing in areas where it has already begun. We have elsewhere noted other cases in which new directions of furrowing set in (Scott, 1946), for example, in the late furrowing of dispermic eggs where the plane of constriction around the neck of a one-quarter blastomere is displaced 45° from the original plane. In the course of another study, as yet unpublished, we observed changes in the patterns made by echinochrome granules located in the presumptive furrow cortex of cleaving *Arbacia punctulata* eggs. We noted that the furrow surface shrinks two-dimensionally during the earliest phases of the cleavage, from the sphere to the cylinder stage, after which it shrinks only around the circumference of the furrow. We speculate, therefore, that the contractile elements of the furrow cortex are arranged at random in the plane of the surface but that effective contraction can only occur in directions where resistance to shrinkage is low. According to this interpretation the contractile elements of the band of furrow cortex are arranged at random in the plane of the surface; during the sphere-to-cylinder stage they all contract whatever their orientation, but during the subsequent stages of the furrowing only those elements oriented around the furrow are able to shorten. This hypothesis can obviously be extended to include the unusual "furrow" patterns resulting from micromanipulation, for example Figures 74–77 and 78–80. In these latter examples, cylinders of furrow substance shrink in directions quite different from the presumptive ones.

Summary

1. Eggs swollen in 60% sea water cleave effectively when flattened.

2. Furrowing continues after abscission of one or both poles of the egg.

3. Furrowing is initiated and completed after hemisection of the still spherical egg through the long axis of the amphiaster.

4. Bisection of the spindle just before egg elongation causes a diastral egg to cleave into four blastomeres, two of which lack asters.

5. Islands of furrow cortex "isolated" at early furrowing shrink progressively during division. This is regarded as evidence for active furrow contraction.

6. Tubes of furrow cortex prepared from the furrow walls shrink in directions quite different from the original one.

7. The furrow cortex is not determined until the beginning moment of elongation for cleavage.

8. The position of the furrow is not necessarily related to the plane of the spindle equator.

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